Accomplishments: 1994-present

1994: Receptor Assays for Domoic Acid and PSP Toxins

New receptor-based assays for domoic acid and PSP toxins have been found to be useful for detecting toxins in toxic algae, shellfish, crab hepatopancreas, and the serum of exposed humans and animals. These high capacity assays are formatted to contain 96 data points on a 3 x 4" filter card to provide rapid, reliable results and detect all toxin congeners in a manner quantitatively proportional to their toxicity. These assays are anticipated to be used in dock side testing and confirmation of marine toxin exposure in humans and marine animals.

Contact: Greg Doucette

1995: Detection of Domoic Acid and PSP Toxin Activity in Algae and Animals

New rapid and inexpensive receptor-based assays for domoic acid and PSP toxins have been found to be reliable for detecting toxins in toxic algae, shellfish, crab hepatopancreas, and the serum and urine of exposed humans and animals. These assays have been validated against HPLC analytical methods and mouse bioassay. National reference laboratories within the European Community have requested that NMFS offer training workshops on implementing the receptor assays and expressed a desire to initiate collaborative testing programs. These assays are anticipated to be used in dockside testing of shellfish and confirmation of marine toxin exposure in seafood consumers. *Contact: Greg Doucette*

1996: Production of PSP Toxins by Bacteria

Recent studies of PSP toxigenesis suggest that both dinoflagellates, as well as certain bacteria associated with these algae, are capable of synthesizing PSP toxins. This has been addressed using both laboratory and field based studies. Reintroduction of bacteria isolated from toxic, but not nontoxic strains of *Alexandrium tamarense* was determined to increase PSP production in axenic *Alexandrium* cultures. Field studies conducted in collaboration with the Department of Fisheries and Oceans (Canada) during a red tide bloom in the lower St. Lawrence River estuary determined that bacteria grown from size excluded isolates produced PSP toxins. Continuing investigations will focus on how bacterial-algal interactions influence PSP toxigenesis.

Contact: Greg Doucette

Development of Reporter Gene Assay for Marine Toxins

A new assay technology has been developed for algal toxins. Reporter gene assays have been established using the c-fos response element linked to the coding region for firefly luciferase and this approach has been published (Analytical Biochemistry). This method is very effective for measuring brevetoxins, PSP toxins and ciguatoxins. The method has a particularly high sensitivity for ciguatoxins and should permit a high capacity monitoring of the toxin in small (<1 g) finfish samples. *Contact: John Ramsdell*

1997: Passage of PSP Toxins Through the Food Web

The PSP receptor binding assay (corroborated by HPLC analyses) is being used to study toxin transfer in Gulf of Maine food webs. Work to date has shown that PSP toxins move preferentially from their algal producers (*Alexandrium* spp.) into the larger size fractions of the zooplankton grazing community dominated by large copepods, even though these animals were not numerically dominant. Toxin-accumulating copepods could provide a direct trophic linkage for vectorial intoxication and possible mortality of plankitvorous fish as well as endangered whales which are known to feed upon these copepods. This project is in collaboration with Dr. D. Anderson (WHOI) and Dr. J. Turner (U. Mass. Dartmouth).

Contact: Greg Doucette

Collaborative Testing of Receptor Assays for Marine Toxins

Receptor based assays for PSP, ASP, NSP, and CFP have been developed and laboratory validation completed in the past four years. These assays are now ready to be tested corroboratively in formal interlaboratory trails. The first of these trails, testing the assay for NSP in lysters, has been initiated as an AOAC Peer Verified Method trial, which will be completed in FY1998. *Contact: Fran Van Dolah*

2000: Accumulation of PSP Toxins in Zooplankton Grazers

Algal toxins are well-known to undergo trophic transfer and accumulation in marine food webs, causing intoxication of upper-level consumers such as fish, sea birds, and marine mammals. Work in collaboration with investigators at U. Mass Dartmouth and the Woods Hole Oceanographic Institution has focused on the tracking movement of PSP toxins from their algal producers into the associated zooplankton grazer assemblage. Through receptor assay-based analysis of algal and zooplankton size fractions from Massachusetts Bay, MA, we determined that PSP toxins did, indeed, accumulate in the grazers and that toxicity was disproportionately concentrated in the larger zooplankton size fractions (200-500 : m, > 500 : m). Interestingly, these size fractions, frequently dominated by large copepods, comprised only a small portion of total zooplankton abundance. These larger toxin-accumulating copepods could thus provide a direct trophic linkage for PSP intoxication of marine mammals such as baleen whales, which are known to preferentially feed upon these grazers.

Contact:Greg Doucette

Recent Advances in and Applications For a PSP Receptor Binding Assay

Several years ago we described a high throughput receptor binding assay for PSP toxins and its use for detecting toxic activity in shellfish and algal extracts. We have since increased the assay efficiency through application of microplate scintillation technology (4h turn around time), and have validated use of 11-[^{3}H]-tetrodotoxin as an alternative radioligand to the [^{3}H]-saxitoxin conventionally employed in the assay. Efforts are now focused on identifying the applications for which the receptor assay can provide data comparable to the more time consuming, technically demanding HPLC analysis of PSP toxins. We have compared the results of both methods for toxic dinoflagellates, field samples of *Alexandrium* spp. and its associated zooplankton grazers, as well as contaminated human fluids from a PSP outbreak. In general, receptor-based STX equiv. values were highly correlated and in close quantitative agreement with those produced by HPLC. While the receptor binding assay does not provide toxin composition data obtainable by HPLC, it does represent a robust and reliable means of rapidly assessing PSP-like toxicity in laboratory and field samples. Moreover, this assay should be effective as a screening tool in suspected cases of PSP intoxication. *Contact: Greg Doucette*

Assay Validation and Technology Transfer

As part of the U.N. sponsored technology transfer program on red tides in SE Asia, we conducted a training workshop on receptor assays in Manila, Philippines in December 1999. The workshop was attended by 14 participants from 7 SE Asian countries. In addition, this year we hosted two individuals associated with this program for extensive receptor assay training in the laboratory: Ms. Cecilia Conaco, of the University of the Philippines (October-Nov 1999) and Ms. Mei Mei Ch'ng, of University of Malaysia (March – August 2000). We will host up to 3 additional personnel from participating nations during FY 2001. The program will then carry out a round robin interlaboratory comparison trial between participating nations in 2002. A receptor assay training workshop was held in May at CCEHBR to transfer this technology to representatives of two state regulatory agencies interested in its potential as a replacement for the mouse bioassay: California Dept. of Health and Florida DNR. *Contact Fran VanDolah*

2001: Initiation of the South Carolina Phytoplankton Monitoring Network

The inaugural year for South Carolina Phytoplankton Monitoring Network began with great enthusiasm and the opening of a new home page http://www.chbr.noaa.gov/CoastalResearch/SCPMN/SCPMNmain.htm. This community outreach program consists of high school marine science and biology classes monitoring local waters for the presence of possible harmful algal species. Teachers participating in the network attended a workshop on algal identification and sampling techniques. Currently, 12 teachers and approximately 170 students are actively sampling local waters for harmful algae. Based on the observations of these groups, a number of potentially harmful species have been detected in South Carolina, some for the first time. These include representatives of the genera *Prorocentrum*, *Pseudo-nitzschia*, *Heterosigma*, and *Akashiwo*. Additional community groups will be added to the network during the next year to extend coverage of this program along the coast of South Carolina. *Contact: Steve Morton*

Toxicogenomics: A Global Approach to Assessing Marine Toxin Exposure and Effects

Toxin exposure almost always causes changes in gene expression, either directly, due to the specific interaction of a toxic agent with its receptor, or indirectly due to the induction of intracellular signaling cascades. Toxicogenomics is the application of DNA arrays to identify a specific pattern of gene expression induced by a particular toxicant. Once a "signature" gene response is identified, this information may be useful for elucidating a toxic mode of action and may potentially yield biomarkers of exposure unique for a particular toxicant or class of toxicants. This year the Marine Biotoxins Program co-organized a workshop on "Toxicogenomics and Nanotechnologies: New Frontiers for Mycotoxins and Phycotoxins" (June 22-23, 2001; Tufts University Bedford, MA) and carried out preliminary studies to determine the suitability of this approach for algal toxin exposure. Changes in gene expression in brains and livers of mice exposed to brevetoxin were studied. Several genes were found to be induced in response to this toxin class. Ongoing studies will determine the dose/response and time course of genetic responses and compare gene induction "signatures" of different algal toxin classes. *Contact: Fran Van Dolah*

2002: cDNA Library Provides Molecular Tools to Understand HAB Formation

Understanding the mechanisms that control the growth and toxicity of dinoflagellates has long been hampered by our lack of insight into their molecular biology, stemming from the lack of molecular tools needed for such investigations. Development and screening of a cDNA library containing expressed gene sequences from the Florida red tide dinoflagellate, *Karenia brevis*, was therefore initiated this year to provide some of these tools. This project has yielded novel insights into the intracellular signaling pathways, cell cycle control, and stress response mechanisms present in this dinoflagellate species. To date, 1150 *K. brevis* expressed sequence tags (ESTs) have been sequenced. Of these, 36% have high homology to known genes in the GenBank database. Using these sequence data, we have developed probes for known cell cycle regulatory proteins to study the mechanisms controlling the growth phase of bloom formation and for stress proteins involved in adaptation/survival of *K. brevis* cells as they are exposed to changing water column conditions. Understanding cellular regulation is a prerequisite to developing truly predictive models or species-specific control strategies.

Contact: Fran Van Dolah

Discovery of PSP Toxins in the North Atlantic Right Whales

Intensive study of the western North Atlantic right whale (*Eubalaena glacialis*) population over the past 20 years has yielded evidence of reproductive dysfunction in this highly endangered cetacean species. Among the factors identified as potentially contributing to this phenomenon, exposure to marine algal toxins has received little consideration. We recently initiated a study to investigate the possible occurrence of paralytic shellfish poisoning (PSP) toxins in *E. glacialis* and in the zooplankton assemblage comprising the majority of its diet. Samples of *E. glacialis* fecal material from at least ten different animals obtained during Aug./Sept. 2001 from the Bay of Fundy, Canada, tested positive for PSP toxins by both receptor binding assay and by HPLC analysis. Zooplankton samples collected during the same time period were also shown to contain similar levels of PSP toxins by weight using both methods. Additional data revealed the presence of toxic *Alexandrium* cells immediately before and during the sampling period. These findings provide the first compelling evidence for the occurrence of PSP toxins in *E. glacialis*, suggesting that further studies are warranted to examine the trophic transfer of these biotoxins via zooplankton vectors and their possible effects on the reproductive success of this endangered species. *Contact Fran Van Dolah*

Transfer of Receptor Assay Technology to SW African Countries Initiated

The southwest African countries of South Africa, Namibia, and Angola have either historical or recently emerging problems with one or more groups of marine algal toxins. These countries have requested assistance through the U.N. International Atomic Energy Agency (IAEA) in establishing capabilities for receptor assay-based detection of algal toxins in seafood products. A project planning meeting was held at IAEA Headquarters in Vienna, Austria to develop a regional technical cooperation proposal for the transfer of the Marine Biotoxins Program's receptor assay technology to each of these three African countries. This project will be modeled after an ongoing IAEA-sponsored program in SE Asia, with the African end-users visiting the CCEHBR laboratory next year for training and returning to their home institutions to begin conducting the assays. An inter-calibration study coordinated through our Program will follow, and then receptor assays will be implemented as a component of their respective toxin monitoring programs, which are either well-established (S. Africa) or currently being developed. Acquisition of receptor-based technology will be of immediate benefit to each of our African partners, given their rapidly growing fishery and aquaculture industries along with the accompanying demands for biotoxin testing of products for export to world markets. *Contact: Fran Van Dolah*

Confirmation of PSP in the Indian River Lagoon: A New Public Health Issue In Florida

Between 1 January – 25 April 2002, 14 pufferfish poisoning incidents in Florida were reported from the Indian River Lagoon. (IRL), Florida. In collaboration with the Florida Marine Research Institute (FMRI), the U.S. FDA, and NRC Canada, we have confirmed the presence of saxitoxins (STX) in pufferfish in the IRL. This is the first toxic event in Florida waters in which STX has been identified. Concentrations of up to 6238 ìg STX eq/100 g were detected in skin, mucus, muscle, and viscera of southern pufferfish, with highest levels in the skin and mucus. STX was also confirmed in conch (*Melongena corona*) and cockle (*Americardia media*) from the IRL, with traces detected in hard clams (*Mercenaria* spp.). Both unialgal cultures of the dinoflagellate *Pyrodinium bahamense* and natural bloom samples (> 3 million cells/L) obtained during fish kills in the IRL tested positive for STX. *Pyrodinium bahamense* var. *bahamense*, the variety found in Florida, has never before been reported to be toxic. Current studies at FMRI are aimed at confirming the origin of the toxic blooms present in the IRL. Recently, the northern IRL has experienced a number of unusual events, including dolphin, manatee, fish, and horseshoe crab mortalities, increased tumor incidence in hard clams, "spicy clams," and reduced natural recruitment and hatchery losses of hard clams. To what extent these events are linked to the emerging issue of toxic *P. bahamense* blooms remains undetermined. Public health risks associated with PSP have resulted in the implementation of new management strategies by the state of Florida, which previously has had to regulate shellfish harvests only for brevetoxins.

Contact: Fran Van Dolah

Volunteers Monitor Harmful Phytoplankton Along South Carolina Coast

The South Carolina Phytoplankton Monitoring Network (SCPMN) began its second year of existence with over 34 groups monitoring state coastal waters for potentially harmful algal species. A total of over 50 sampling sites from all coastal counties of South Carolina are monitored each week. Volunteer groups are composed of both middle and high school students, state park personnel, and citizen environmental groups. This NOAA sponsored community program serves to increase the awareness of constituent groups about the many issues related to harmful algae and directly involves volunteers in coastal stewardship. In the SCPMN's first year of existence, volunteers observed three potentially toxic algae, including *Pseudo-nitzschia*, *Dinophysis*, and *Prorocentrum lima*. Observation and identification of phytoplankton along the South Carolina coast will be useful in developing a species list and record of distribution, as well as alerting NOAA scientists to the presence of potentially harmful species at the many sampling sites. *Contact: Steve Morton*

2003: Purification of a Novel Toxic Compound Produced by *Alexandrium monilatum* Isolated from the Gulf of Mexico

Since the 1930's, the chain-forming dinoflagellate, *Alexandrium monilatum,* has formed blooms in coastal waters of all Gulf states and has frequently been associated with fish mortality events.. In 1967, research chemists described a cytotoxic, lipophilic compound derived from extracts of this species; however, the purification and structure of this toxic substance(s) was never reported. A strain of *A. monilatum* isolated from a red tide off Mississippi (strain AM01) was grown in batch culture to mid-log phase and examined for the possible production of bioactive compounds. The harvested cell mass was extracted using an elutropic series of increasing polarity using 5 different solvents and each fraction was tested for activity using different live animal and-cell based assays. Two distinctive toxic fractions were observed: a polar soluble fraction and a non-polar soluble fraction. Our current effort has focused on isolating and determining the chemical structure of the non-polar soluble compound, beginning with chromatographic separations. Subsequent mass spectral analysis using both LC-MS and MALDI-MS of the resulting purified compound yielded a molecular ion of 790 amu. Proton and carbon NMR structural analysis demonstrate a macrolide-like compound with four exo-cyclic double bonds. This novel compound has a nominal molecular formula of $C_{47}H_{98}O_8$. *Contact: Steve Morton/Peter Moeller*

Collaboration with Naval Research Laboratory Successfully Tests Portable Biosensor for Toxic Algae

On-line, near-real time detection systems for harmful algal species and their toxins is a rapidly emerging field aimed at forecasting bloom development, persistence, and toxicity as well as providing data to facilitate rapid and more effective responses to harmful algal blooms. Recently the Naval Research Laboratory demonstrated that cultured neuronal networks grown over microelectrode arrays (MEAs) are capable of detecting brevetoxins, saxitoxin, and domoic acid. An on-site collaboration at the Marine Biotoxins Program using a prototype portable battery-operated unit containing a central core of living neurons growing on a biosensor chip sought to determine if the sensor could detect these toxins directly in the seawater growth medium of Alexandrium fundyense and Karenia brevis The instrument responded with positive toxin signatures from the sonicated medium of each red tide alga, but not from non-toxic isolates of the same algal genus. This successful trial provided evidence that the prototype MEA has the capacity to detect toxins associated with cells of toxic algal species and exhibits the potential for monitoring toxin levels during harmful algal blooms. *Contact: John Ramsdell*

Optimization and Inter-laboratory Comparison of PSP Receptor Assay Completed with California Dept. of Health Services

The technology for a rapid, cost-effective receptor binding assay for paralytic shellfish poisoning (PSP) toxins developed by CCEHBR scientists was transferred to colleagues at the California Department of Health Services (CDHS). Due to differences in instrumentation between the two laboratories, optimization of the assay at CDHS was required, which was followed by an inter-laboratory comparison of results. Precision, accuracy, and sensitivity (LOD = 0.2 µg STX eq./100 g shellfish tissue) of the CDHS-modified protocol were equivalent to those of the original method. Determination of PSP toxin concentrations in shellfish samples collected through the CDHS monitoring program agreed closely between the two laboratories (within ~10%), demonstrating the robustness and adaptability of the assay. This technology shows a very high potential for replacing the currently employed mouse bioassay (MBA), which has drawn increasing criticism due to its use of live animal testing. At present, the receptor assay can serve as a rapid, high throughput screen prior to testing by MBA and provide an early warning of increasing PSP toxicity when toxin levels are below the MBA limit of detection.

Contact: John Ramsdell

Characterization of PSP Toxin Trophic Transfer Through Zooplankton Grazers in the Gulf of Maine

The initial vectors involved in transferring PSP toxins from their algal producers to higher trophic levels are important in determining the ecosystem components ultimately affected by these potent neurotoxins. Since zooplankton frequently serve as this initial vector, CCEHBR scientists, in collaboration with colleagues at U. Mass. Dartmouth and the Woods Hole Oceanographic Institution, are studying entry of PSP toxins into the grazer community in order to identify the primary routes of trophic transfer in the Gulf of Maine. Results to date indicate that PSP toxins can accumulate in grazer size fractions ranging from 64 to >500 µm, with the distribution of toxin changing as a function of the zooplankton species present and their abundance, as well as the amount of toxin contained within the *Alexandrium* cells being grazed. PSP toxin entry into the larger zooplankton size fractions was observed during late spring/early summer, which has important implications for making these toxins available to cetaceans that actively feed on copepods in this region over the same time period.

Contact: Greg Doucette

Success of Prototype Remote Harmful Algal Bloom (HAB) Sensor Stimulates Development of Second Generation Instrument

The first generation of an autonomous, *in situ* sensor for HAB species and their toxins, called the Environmental Sample Processor (ESP), has been field tested successfully for domoic acid-producing *Pseudo-nitzschia* and for saxitoxin-producing *Alexandrium*. As part of a collaborative project under the multi-agency National Oceanographic Partnership Program (NOPP), scientists from CCEHBR and the Monterey Bay Aquarium Research Institute (MBARI) are now designing a second generation ESP platform that will be smaller and include enhanced sampling/processing capabilities that will increase its flexibility for use in monitoring and research. Membrane-based arrays will provide concurrent, near-real time detection for both organisms and toxins. Data telemetered autonomously from the ESP to land-based facilities will aid in efforts to forecast bloom development and movement, and will ultimately be available through a web-based user interface.

Contact: Greg Doucette